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Genetic Variation Assessment of Some Prunus Species Using Srap Markers

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Abstract:

Genetic variation at molecular level was evaluated among three species of Prunus genus and a wild species, (P. arabica (wild), P. argentea (wild), P. dulcis (local), and P. dulcis (wild). The genetic variation assessment was carried out using SRAP molecular markers. The genetic similarity coefficient revealed the genetic relationship among the samples tested in which the highest genetic distance was between P. dulcis (local), and P. dulcis (wild), and lowest genetic distance was between P. arabica (wild), P. argentea (wild). The phylogenetic tree was obtained using UPGMA method depending on the total number of SRAP bands. There were two main groups in the dendrogram: the first one consists of two subgroup: P. arabica and P. argentea cluster together in one Subgroup and P. dulcis (wild) appear alone in this subgroup. P. dulcis (Local variety) appear in the second group alone.

KEYWORDS: *SRAP marker, Prunus species, genetic diversity.*

Introduction:

The important of evaluation the level of genetic diversity in natural populations of a species are the key role for the plants breeders and genetic resource conservation programs (Cohen et al., 1991). The wide variability of wild species in terms of phenological, morphological, abiotic, biotic, and quality traits, they play crucial roles in breeding programs (Laidò et al., 2013). It has been widely reported that a large amount of genetic diversity has been lost in major crops due to genetic drift and selection in comparison with the wild forms, thereby reducing the potential for crop improvement in modern agricultural systems (Evans 1997). Genetic variation must exist to maintain natural populations as evolutionarily viable units capable of adapting to changing environmental conditions in the long term (Sreekanth et al., 2012). Thus, a genetic resource management strategy should involve an investigation of the genetic diversity and the extent of genetic differentiation within and between populations (Rao and Hodgkin, 2002). In this work, we present a members of the genus Prunus belonging to the family Rosaceae, (P.arabica (wild), P.argentea (wild), P.dulcis (local), and P.dulcis var. amara (Wild.) which represent some natural population growing in this region to determine some accurate evaluation of the naturally occurring polymorphism. For the characterization and evaluation of genetic diversity among different plant species and population many molecular markers has been used to detect many character and evaluation of genetic diversity. (Graham et al., 2004; Fu et al., 2006; Sargent et al., 2007; Lewer et al., 2008; Ahmed et al., 2009). There are very limit number of scientific articles describing the genetic variation of this species, and if any reports are a viable most likely would be characterization of these species interns' morphological characteristics.

In this present study we used Sequence-related amplified polymorphism (SRAP) markers which have been recognized as useful molecular markers for diversity studies, population genetic analysis, and other purposes in various species.

Materials and Methods

Dna Extraction:

Samples of Prunus species leaves were collected from villages around Duhok city (near Duhok Dam) (P.arabica, P.argentea P.dulcis (local), and P.dulcis var. amara). The samples Genome DNA was extracted from leaf tissues by CTAB according to the method by Waigand et al., (1993).

Srap Marker Testing

The PCR mixture consists of 50 ng/ µl of DNA template, 5 pmol of each primer, 10× PCR Buffer, 0.25 mM MgCl2, 0.5 mM dNTPs, 1U/ µl Tag DNA polymerase in a total volume of 20 µl. The amplification profile was: an intiail denaturation step of 5 min at 94□, followed by five cycles of denaturation 94℃ for 1 min, annealing 35℃ for 1 min, and elongation 75℃ for 1min; and followed by thirty five cycles of 94□ for 1 min, annealing 50℃ for 1 min, and elongation 72℃ for 1 min, the final extension at 72℃ for 5 min. The PCR products (2.5 µl) were separated by electrophoresis on a 1.5% agarose gel at 60 W constant powers for 2h.

SRAP COMBINATIONS PRIMERS:

The primers combinations used in this study listed in Table (1).

Table (1): Represents the forward and reverse sequences of these primers.

	Reverse	Forward	5' ⟶ 3'
EM1	GACTGCGTACGAATTAAT	ME4	TGAGTCCAAACCGGACC
EM15	GACTGCGTACGAATTCTG	ME1	TGAGTCCAAACCGGATA
EM15	GACTGCGTACGAATTCTG	ME13	TGAGTCCAAACCGGCAT
EM15	GACTGCGTACGAATTCTG	ME12	GGTGAACGCTCCGGAAG
EM16	GACTGCGTACGAATTCGG	ME9	TGAGTCCAAACCGGTCA
EM16	GACTGCGTACGAATTCGG	ME10	TGAGTCCAAACCGGAAA
EM16	GACTGCGTACGAATTCGG	ME11	TGAGTCCAAACCGGAAC
EM16	GACTGCGTACGAATTCGG	ME1	TGAGTCCAAACCGGATA
EM16	GACTGCGTACGAATTCGG	ME2	TGAGTCCAAACCGGAGC
EM16	GACTGCGTACGAATTCGG	ME4	TGAGTCCAAACCGGACC
EM17	GACTGCGTACGAATTCCA	ME1	TGAGTCCAAACCGGATA
EM17	GACTGCGTACGAATTCCA	ME2	TGAGTCCAAACCGGAGC

DATA ANALYSIS

The PCR amplified products were scored as 1 or 0 respectively for the presence or absence of bands across the genotypes to generate a binary matrix. The binary matrix was analyzed using the NTSYS-PC version 2.10 software to calculate the similarity values and to generate the phylogram. Similarity coefficient was calculated using the software NTSYS-PC version 2.10 (Nei, 1978). Cluster analysis was conducted on similarity using the un-weighted pair group method on arithmetic averages (UPGMA).

RESULTS AND DISCUSSION:

Sixteen SRAP combinations were used for the study the genetic diversity among the selected Prunus species used in this study.

Genetic similarity represent coefficient matrix of the these Prunus species based on the data of the sixteen combinations SRAP primers Showed in Table (3), the highest genetic distance were between P.dulcis (local) and P. dulcis (wild), and lowest genetic distance were between P. arabica and P .argentea.

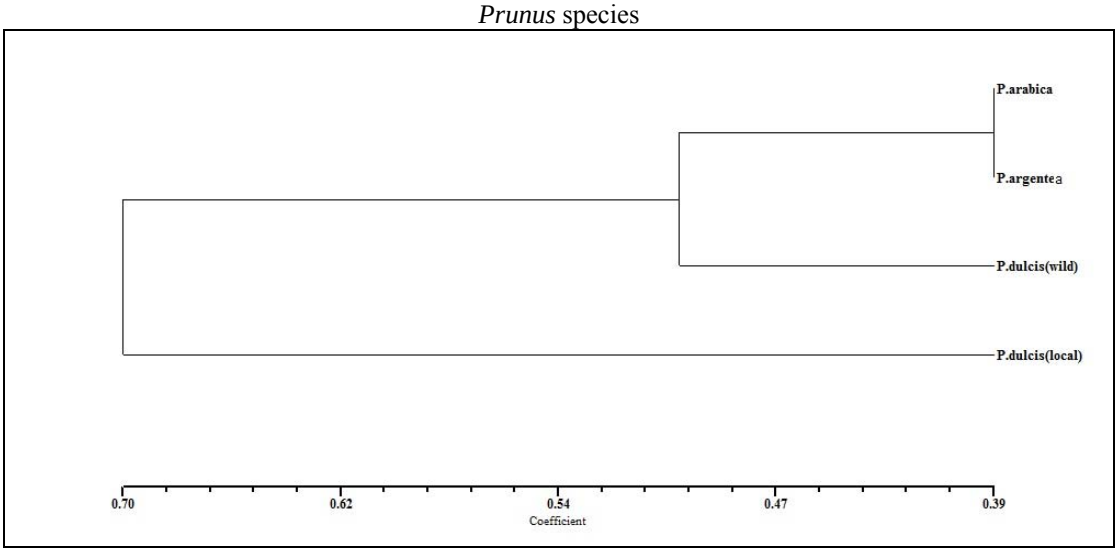
Table (3): present genetic similarity coefficient matrix of the some Prunus species:

	P. arabica	P. argentea	P. dulcis (local)	P.dulcis (wild)
P. arabica	0.0000			
P .argentea	0.3869	0.0000		
P. dulcis	0.7848	0.4505	0.0000	
P dulcis (wild)	0.4827	0.5187	0.8684	0.0000

CLUSTER ANALYSIS:

A dendrogram was obtained by the UPGMA method using the total number of SRAP bands (Fig. 1). There were two main groups in the dendrogram: the first one consists of two subgroup: P. arabica and P. argentea cluster together in one Subgroup and P. dulcis (wild) appear alone in this subgroup. P. dulcis (Local variety) appear in the second group alone.

Figure (1): A dendrogram Neighbor–joining tree representing the genetic relationships among some selected *Prunus* species



Aradhya et al. (2004) they analysed genetic variability and differentiation within and among seven cultivated species and seven wild species of Prunus using AFLP marker, they reported that the wild species clustered together and the cultivated species appear together. These results agree with results obtained in this research, which suggested that wild P. arabica and P. argentea have a relatively high level of genetic diversity; which was attributed to their being less affected by human disturbance

The SRAP marker system is becoming the marker of choice for characterization and genetic diversity studies in a wide range of plants. The study described in this paper shows that SRAP analysis is a powerful tool also for the characterization of genetic diversity of Prunus species.

In conclusion, these results obtained by SRAP analysis were in general agreement with morphological classification, suggesting that SRAP is a simple and effective molecular marker technique and could be successfully applied to the study of genetic relationships, and to plant breeding. Our results also suggest that both morphological and molecular tools should be used for the classification of the genus Prunus.

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ئاشکەراکرن گوهورینێن بوماوەیی بو هندەك جوراندا بو توخمێ Prunus بکارئینانا تەکنیکارییّن SRAP

کورتیا لێکولینێ:

ئەڤ ڤەکولینە هاتە ئەنجامدان ژبو ئاشکەراکرنا وخەملاندنا گوهورینێن بوماوەیی دناڤبەرا هندك جورێن ژ توخمێ Prunus . بکارئینانا arabica (wild), P. argentea (wild), P. dulcis (local), , and P. dulcis (wild نیشاندەرێن SRAP.

ئەنجامێن شلوڤەکرنا دوریا بوماوەیی دیارکر کو نێزیکبوونەکا بوماوەیی یا P. dulcis (wild و dulcis (local) دناڤبەرا خودا دیارکر ب دورترین دوریا بوماوەیی دناڤبەرا واندا لێ هەردوو جورێن (P. arabica (wild), P. argentea wild) نێزیکبوونەکا بوماوەیی ب کێمترین سەرجەمێ بوماوەیی دیارکر. شلوڤەکرنا دارا بوماوەیی ییّن پێکڤەگرێدای دەرکەتنا دوو لقێن سەرەکی دیاربو، لقێ ئێکێ، دوو لقێن دووەمی بخۆڤە دگریت وئەڤ جورە تێدانە (P.dulcis) P. argentea و (wild P. arabica (wild), . لقێ دووێ یێ سەرەکی ئەڤ جورە (P. dulcis (Local) تیدایە.

تقدير التغايرات الوراثية لبعض انواع جنس ال Prunus باستخدام تقنية ال SRAP

الخلاصة:

تم تحليل التغاير الوراثي لثلاثة انواع من جنس Prunus وهي P. arabica (wild), P. argentea (wild), P. dulcis (local), , and P. dulcis (wild لكشف وتقدير التغايرات الوراثية بين هذه الأنواع باستخدام تقنية SRAP. أظهرت نتائج تحليل البعد الوراثي أن P. dulcis (local), , and P. dulcis (wild أظهرا قرابة وراثية بأعلى بعد وراثي بينهما بينما اقل بعد وراثي كان بين(wild) P. arabica (wild), P. argentea . كما اظهرتالشجرة الوراثية المترابطة والتحليل التجميعي الى مجموعتين رئيسيتين، المجموعة الاولى تضمنت مجموعتين ثانويتين الاولى تضمنت P. arabica و P. argentea اما P.dulcis (wild) فقد ظهر لوحده في المجموعة الثانوية الثانية. المجموعة الرئيسية الاخرى فأن P. dulcis (Local) فقد ظهر لوحده في هذه المجموعة.